

Original Research Article

Diosgenin ameliorates lipopolysaccharide-induced acute kidney injury through modulation of oxido-inflammatory responses and attenuation of pyroptosis and mitochondrial dysfunction

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Abstract

Objective: Sepsis-associated acute kidney injury (AKI) involves interconnected disturbances in renal redox balance, inflammatory signaling, and inflammatory cell death, including pyroptosis. While oxidative stress is a known contributor, the roles of the NLRP3 inflammasome and the pro-inflammatory cytokine interleukin 17 (IL-17) in this pathology are of growing interest. Here, we examined whether diosgenin could counteract renal injury produced by systemic lipopolysaccharide (LPS) exposure, with particular emphasis on inflammatory and pyroptotic signaling.

Materials and Methods: Thirty-two male C57BL/6 mice were randomly divided into four groups (n=8 per group): Control, Control + Diosgenin (40 mg/kg), LPS (10 mg/kg), and LPS + Diosgenin (40 mg/kg). Diosgenin was administered via oral gavage 1 hr prior to a single dose of LPS injection (i.p.). After 24 hr, renal function was assessed in terms of serum creatinine, blood urea nitrogen (BUN), and cystatin C levels. Oxidative stress markers (malondialdehyde (MDA), superoxide dismutase (SOD), and nitrite) were also measured. In addition, renal levels of NLRP3, caspase-1, and IL-17 were determined. Finally, myeloperoxidase (MPO) activity and mitochondrial membrane potential (MMP) were assessed.

Results: Pretreatment with diosgenin mitigated the functional renal impairment induced by LPS, as reflected by lower levels of creatinine, BUN, and cystatin C and reduced oxidative stress (lower MDA and higher SOD). It also suppressed key inflammatory mediator IL-17 and pyroptotic NLRP3/caspase-1 pathway, MPO activity, and mitochondrial dysfunction.

Conclusion: These findings indicate that diosgenin confers renoprotection against LPS-induced AKI that is associated with attenuation of oxidative stress, IL-17-related inflammatory responses, NLRP3/caspase-1 signaling, and preservation of MMP. Collectively, these results support the potential of diosgenin as a promising multi-target protective agent in sepsis-associated AKI.

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Introduction

Acute kidney injury (AKI) refers to an abrupt, often reversible decline in glomerular filtration, clinically recognized through a fast-rising serum creatinine alongside diminished urine output (Xin *et al.* 2024). Its causes are diverse and include renal ischemia and hypoperfusion, direct nephrotoxic exposure, and most frequently systemic inflammatory states such as sepsis. Regardless of the trigger, these insults converge on tubular epithelial cell death and necroinflammation, setting off a repair process that is frequently dysregulated (Zarbock *et al.* 2014). It is noteworthy that functional impairment in AKI may progress to structural injury that remains undetectable by conventional biomarkers. Moreover, recent evidence indicates that inadequate or delayed management during the acute phase can contribute to long-term consequences including chronic kidney disease, cardiovascular complications, and even an elevated risk of renal malignancies (Veltkamp *et al.* 2025). The incidence of AKI has risen steadily over the past few decades. Despite advances in clinical management and supportive care, AKI remains a major global health burden, particularly in intensive care units and resource-limited settings, with mortality rates surpassing those associated with heart failure or diabetes. More importantly, the long-term consequences of an AKI episode extend well beyond the initial injury (Jiang *et al.* 2019). Since no definitive therapy exists for AKI, preventive strategies are crucial to mitigate its onset and their implementation can markedly reduce mortality, hospital stay duration, and overall healthcare costs.

To model the inflammatory cascade underlying sepsis-associated AKI, experimental studies commonly rely on lipopolysaccharide (LPS) administration. As a principal component of the Gram-negative bacterial outer membrane, LPS is one of the most frequently employed inducers of sepsis and AKI in animal

research (Plotnikov *et al.* 2018). Upon engaging Toll-like receptor 4 (TLR4) (in complex with MD2/CD14), LPS activates both MyD88- and TRIF-dependent signaling branches, converging on nuclear factor kappa-light-chain-enhancer of activated B cells (NF- κ B) and mitogen-activated protein kinase (MAPK) activation. This drives substantial pro-inflammatory cytokine output, excessive reactive oxygen species (ROS) generation, and eventual tubular injury and death an axis central to the development of sepsis-associated AKI (Anderberg *et al.* 2017; Nijland *et al.* 2014). The end result is loss of tubular architecture, disruption of nephron integrity, and marked functional decline, a sequence widely regarded as a core driver of sepsis-induced renal injury (Heyman *et al.* 2011).

The pathogenesis of LPS-induced AKI is defined by a pathogenic synergy between oxidative stress, inflammation, and the execution of inflammatory cell death, with pyroptosis emerging as the critical terminal effector pathway. LPS binding to TLR4 initiates canonical inflammatory signaling e.g. NF- κ B and induces ROS generation, creating a milieu that primes and activates the NLRP3 inflammasome. This complex serves as the molecular platform where oxidative and inflammatory signals converge, leading to caspase-1 activation (Wu *et al.* 2025). Caspase-1-mediated cleavage of gasdermin D (GSDMD) is the hallmark step of pyroptosis, producing membrane pores, and inducing lytic death, and release of mature interleukin 1 β (IL-1 β) and interleukin 18 (IL-18) (Xiao and Li 2024). Beyond directly eliminating renal parenchymal cells, this process fuels sterile inflammation via damage-associated molecular pattern (DAMP) release, generating a self-reinforcing injury loop (Wu *et al.* 2022). Consequently, targeting the pyroptotic cascade, particularly at the level of inflammasome activation or Gasdermin D (GSDMD) pore formation, represents a

promising therapeutic frontier in septic AKI (Li et al. 2023).

Diosgenin - 25R-spirost-5-en-3 β -ol- is a naturally occurring steroidal saponin found across several medicinal plants, among them are wild yam (*Dioscorea spp.*), eggplant, raspberry, costus, and fenugreek (Pandey et al. 2025). Beyond serving as a precursor for steroid hormone synthesis, it has attracted sustained research attention for its broad antioxidant (Khateeb et al. 2022), anti-inflammatory (Karami-Mohajeri et al. 2022), anticancer, and tissue-protective properties. Numerous studies have demonstrated that diosgenin mitigates oxidative stress and preserves tissue integrity by enhancing cellular antioxidant defenses, reducing lipid peroxidation, and inhibiting ROS generation (Khateeb et al. 2022; Xiang et al. 2024). It also dampens inflammation and programmed cell death through inhibition of NF- κ B/MAPK signaling and by modulating tumor necrosis factor α (TNF- α), IL-1 β , IL-6, BAX, BCL-2, and Nrf2 expression (Karami-Mohajeri et al. 2022; Leng et al. 2020; Park et al. 2025). The combination of these effects positions diosgenin as a promising candidate for both therapeutic and preventive interventions in conditions where oxidative stress and inflammation are central pathological factors including AKI. Building on this rationale, the present study examined whether diosgenin could limit renal oxidative stress, inflammation, and pyroptosis in a murine model of LPS-induced AKI.

Materials and Methods

Ethical approval

All procedures received prior approval from the Ethics Committee of Shahed University (IR.SHAHED.REC.1396.58) and adhered to the NIH Guidelines for the Care and Use of Laboratory Animals. Every effort was made to minimize animal distress and to keep group sizes to the minimum needed for statistical validity.

Animals

Male C57BL/6 mice weighing 20–25 g were supplied by the Razi Vaccine and Serum Research Institute (Iran). Following a one-week acclimatization period under stable housing conditions (23 $\pm$ 1°C, 45–50% relative humidity, 12-hr light/dark cycle), animals had unrestricted access to standard chow and water throughout the study.

Experimental procedure

A total of 32 mice were randomly assigned to four groups of eight: control, control plus diosgenin (40 mg/kg), LPS, and LPS plus diosgenin (40 mg/kg, p.o.). AKI was induced by a single intraperitoneal injection of LPS from *Escherichia coli* (10 mg/kg; Cat. No. L2630, Sigma-Aldrich, USA) dissolved in saline, using a dose selected based on prior work establishing this model (Chen et al. 2015). Diosgenin (Sigma-Aldrich, USA), suspended in carboxymethyl cellulose, was administered orally by gavage at 40 mg/kg one hour before LPS injection, following the treatment schedule used in that earlier report (Chen et al. 2015). Twenty-four hours after LPS administration, animals were deeply anesthetized with ketamine-HCl (150 mg/kg, intraperitoneal, i.p.), after which cardiac blood was collected, and the kidneys were promptly harvested for downstream analyses.

Blood biochemical assessment

Blood samples were allowed to clot and then centrifuged at 3,000 rpm for 1 min to obtain serum. Creatinine, BUN, and cystatin C, as core indices of renal function, were quantified using commercial assay kits (Pars Azmun Co., Tehran, Iran) according to the manufacturer's instructions.

Measurement of renal oxidative stress-associated indices

Kidney samples were homogenized (10% w/v) in cold lysis buffer using a rotary homogenizer (IKA Co., Germany). The

homogenates were centrifuged, and the resulting supernatants were stored at -70°C until further analysis.

Malondialdehyde (MDA) content was measured as previously described (Nasri et al. 2011; Sedaghat et al. 2014). Briefly, the supernatant was mixed with trichloroacetic acid and thiobarbituric acid, heated in a boiling water bath for 90 min, cooled, and centrifuged at 1,000 rpm for 1 min. The absorbance of the resulting supernatant was read at 532 nm, and MDA concentration was calculated using a tetraethoxypropane standard curve.

Superoxide dismutase (SOD) activity was assessed according to established protocols (Kiasalari et al. 2013; Mirshekar et al. 2010). The supernatant was incubated with xanthine and xanthine oxidase in potassium phosphate buffer (pH 7.8) at 37°C for 40 min, followed by addition of nitroblue tetrazolium (NBT). Blue formazan formation was monitored at 550 nm, and one nitrite unit was defined as the amount of protein required to inhibit NBT reduction by 50%.

Protein concentration was determined by the Bradford assay using bovine serum albumin as the standard (Bradford, 1976).

Nitrite concentration was measured using the Griess reaction (Zhang et al. 2000). In brief, the supernatant was mixed with Griess reagent (sulfanilamide and N-naphthyl-ethylenediamine under acidic conditions), and the absorbance of the resulting chromophore was read at 540 nm.

Measurement of renal IL-17, NLRP3, and caspase-1 levels by ELISA

Renal tissue levels of IL-17, NLRP3, and caspase-1 were measured using commercial ELISA kits (IL-17: Cat # sc-52567, Santa Cruz Biotechnology; NLRP3: Cat # MBS7606270, MyBioSource; and caspase-1: Cat # AB273268, Abcam), with absorbance read at 405 nm on a microplate reader.

Assessment of mitochondrial membrane potential (MMP)

Mitochondrial permeability transition is a key early event in apoptosis, during which disruption of the mitochondrial electrochemical gradient ($\Delta\Psi$) occurs following pore formation induced by activated Bax, Bid, Bak, or Bad. This event promotes the release of cytochrome c and subsequent caspase activation. Mitochondrial membrane potential (MMP) was assessed as an overall indicator of mitochondrial integrity. Briefly, mitochondrial fractions were isolated by centrifugation at 10,000 rpm, and the resulting pellet was incubated with Rhodamine 123 (Cat # R8004, Sigma-Aldrich, USA). Fluorescence intensity was then recorded at 488 nm (excitation) and 525 nm (emission) to quantify MMP (Samodelov et al. 2024).

Determination of myeloperoxidase (MPO) activity

MPO activity was measured in kidney homogenate prepared in a pH 4.7 buffer, followed by centrifugation and repeated freeze–thaw cycles of the pellet to liberate the enzyme. Activity was calculated from the absorbance change at 450 nm in the presence of tetramethylbenzidine (1.6 mM) and hydrogen peroxide (0.3 mM), with the reaction stopped by adding 50 μL of 4 M sulfuric acid (Húngaro et al. 2020).

Statistical analysis

Data were analyzed using GraphPad Prism (v9.5) and are reported as mean $\pm$ SEM. Normality was assessed with the Shapiro–Wilk test, and between-group differences were evaluated using one-way ANOVA with Tukey's post hoc comparison; $p < 0.05$ was considered significant.

Results

Renal function biomarkers

The beneficial effect of diosgenin pretreatment on renal function markers was evaluated. As presented in Figure 1A, serum BUN levels exhibited a marked and

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highly significant elevation in the LPS group compared with the control group ($p < 0.001$). Pretreatment with diosgenin (40 mg/kg) significantly attenuated this rise ($p < 0.05$ vs. LPS). Similarly, serum cystatin C concentrations (Figure 1B) were profoundly increased following LPS administration relative to the control group ($p < 0.001$), whereas diosgenin pretreatment produced a modest yet significant reduction

($p < 0.05$ vs. LPS). In addition, serum creatinine levels (Figure 1C) were strongly elevated in response to LPS challenge ($p < 0.001$ vs. control). Notably, diosgenin pretreatment significantly decreased creatinine concentrations ($p < 0.01$ vs. LPS). Together, these findings indicate that diosgenin effectively mitigates LPS-induced renal functional impairment.

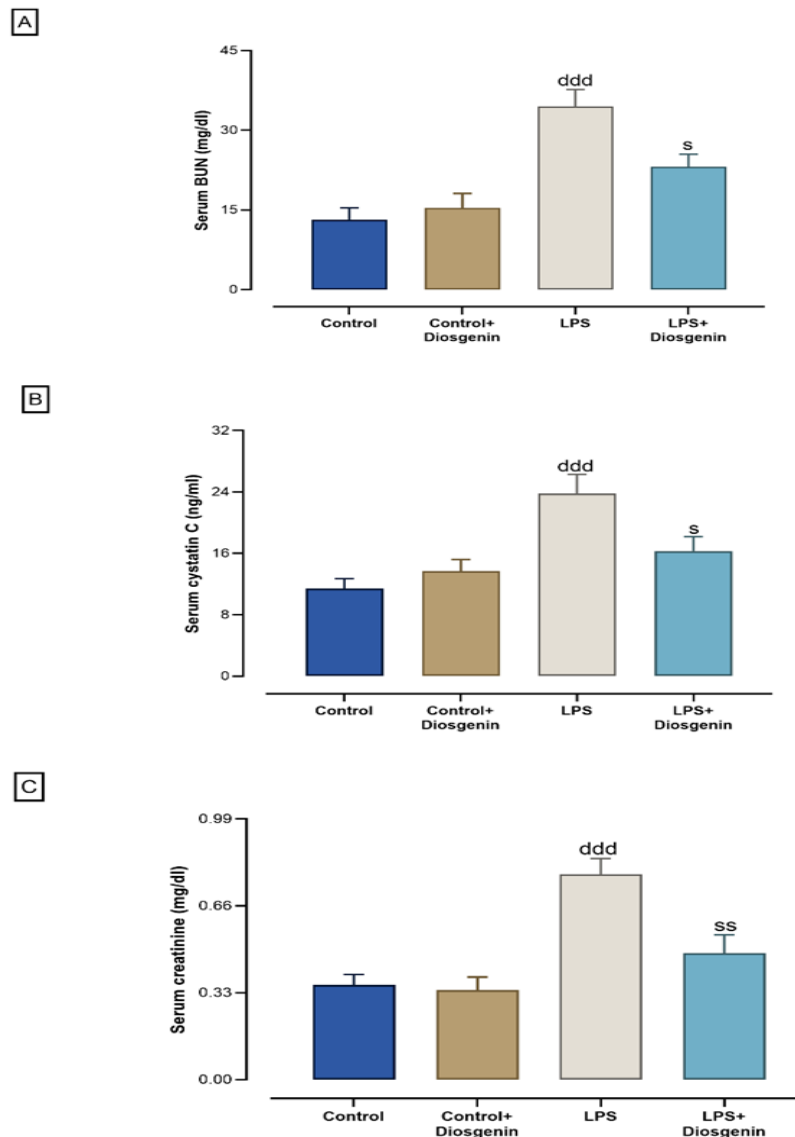


Figure 1. Effects of diosgenin on renal function biomarkers in LPS-induced AKI. Serum levels of (A) BUN, (B) cystatin C, and (C) creatinine in control, LPS-treated, and diosgenin-pretreated groups. LPS administration markedly elevated all renal function markers, whereas diosgenin (40 mg/kg) significantly attenuated these increases. Data are presented as mean $\pm$ SEM. Statistical significance: ddd $p < 0.001$ vs. control; s $p < 0.05$ and ss $p < 0.01$ vs. LPS.

Renal oxidative stress markers

LPS administration led to pronounced alterations in renal oxidative stress parameters. Specifically, MDA levels were markedly increased in the LPS group compared with the control group ($p<0.001$; Figure 2A). Similarly, the level of nitrite showed a significant elevation ($p<0.05$; Figure 2B). In contrast, the activity of SOD was significantly reduced following LPS exposure ($p<0.001$; Figure 2C).

Pretreatment with diosgenin (40 mg/kg) substantially ameliorated these disturbances, as evidenced by a significant reduction in the level of MDA ($p<0.05$ vs. LPS; Figure 2A) and a significant restoration of SOD activity ($p<0.05$ vs. LPS; Figure 2C). Although diosgenin resulted in a downward trend in the level of nitrite, this effect did not reach a statistical significance (Figure 2B).

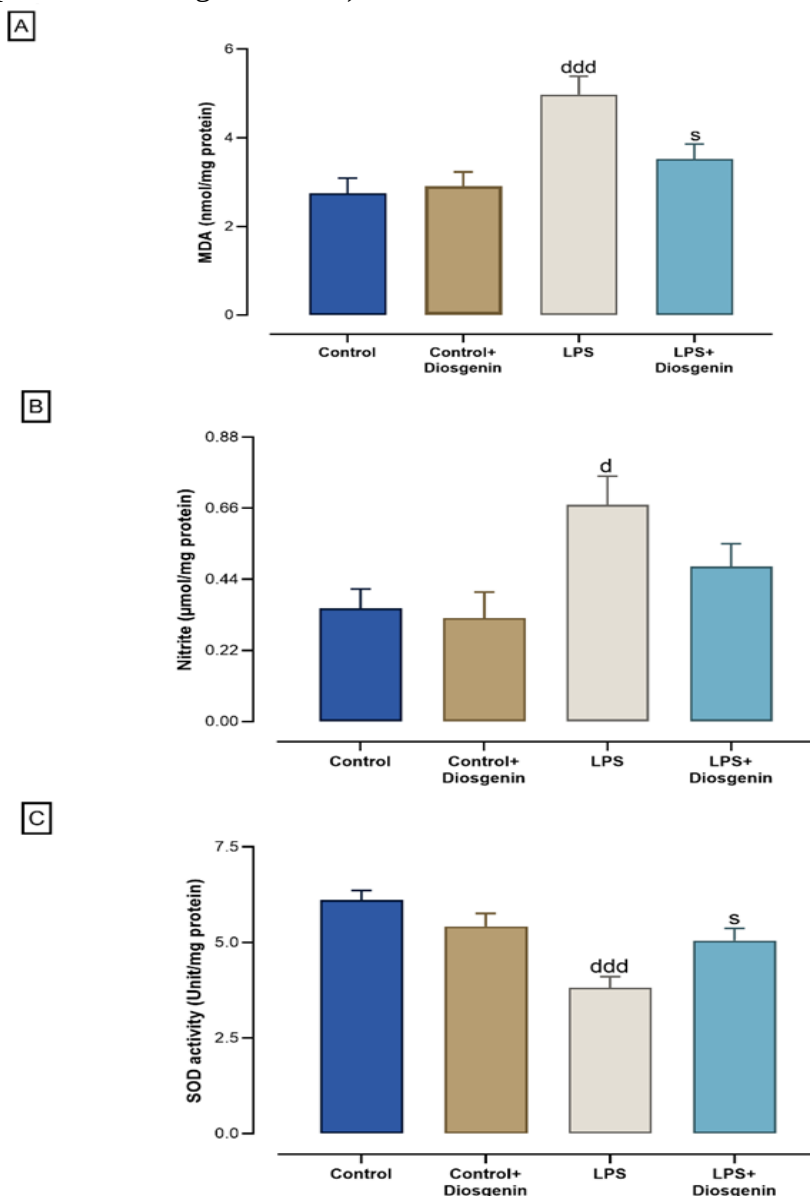


Figure 2. Effects of diosgenin on renal oxidative stress indices following LPS challenge. Renal level of MDA (A), level of nitrite (B), and activity of SOD (C) across experimental groups. LPS induced a significant oxidative imbalance, characterized by increased MDA and nitrite levels and reduced SOD activity. Diosgenin pretreatment significantly normalized MDA levels and restored SOD activity, with a non-significant decreasing trend observed for nitrite. Data expressed as mean $\pm$ SEM. Statistical significance: ddd $p<0.001$, d $p<0.05$ vs. control; s $p<0.05$ vs. LPS.

Inflammatory-related parameters

Regarding inflammatory mediators, LPS challenge induced a robust inflammatory response, as reflected by a significant elevation in MPO levels ($p < 0.001$; Figure 3A), and IL-17 levels ($p < 0.001$; Figure 3B) compared with the control group. Pretreatment with diosgenin (40 mg/kg) markedly attenuated these inflammatory alterations, resulting in a significant reduction in MPO ($p < 0.05$ vs. LPS; Figure 3A) and IL-17 levels ($p < 0.05$ vs. LPS; Figure 3B).

Pyroptosis-related indices

LPS administration resulted in a marked dysregulation of pyroptosis balance, as indicated by a pronounced increase in caspase-1 levels ($p < 0.001$; Figure 4A) and NLRP3 levels ($p < 0.001$; Figure 4B).

compared with the control group. Pretreatment with diosgenin (40 mg/kg) significantly ameliorated these alterations, evidenced by a notable decrease in caspase-1 ($p < 0.05$ vs. LPS; Figure 4A) and NLRP3 levels ($p < 0.01$ vs. LPS; Figure 4B).

Mitochondrial membrane potential (MMP) assessment

MMP serves as a critical indicator of mitochondrial health and function. LPS administration induced significant mitochondrial dysfunction, evidenced by a marked reduction in MMP level ($p < 0.01$; Figure 5) compared to the control group. Pretreatment with diosgenin (40 mg/kg) effectively attenuated this dysfunction, leading to a significant restoration of MMP level ($p < 0.05$ vs. LPS; Figure 5).

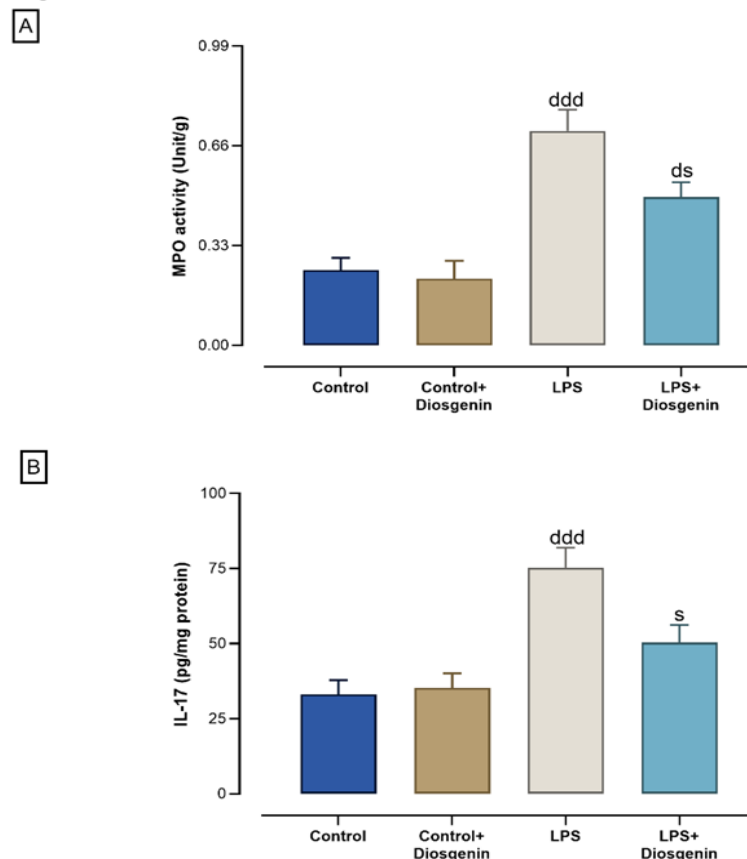


Figure 3. Effects of diosgenin on renal inflammatory mediators in LPS-induced AKI. Renal level of MPO (A) and IL-17 (B) in control, LPS, and diosgenin-treated animals. All inflammatory markers exhibited profound elevations in the LPS group. Diosgenin pretreatment significantly reduced these inflammatory responses. Values shown as mean $\pm$ SEM. Statistical significance: ddd $p < 0.001$ and d $p < 0.05$ vs. control; s $p < 0.05$ vs. LPS.

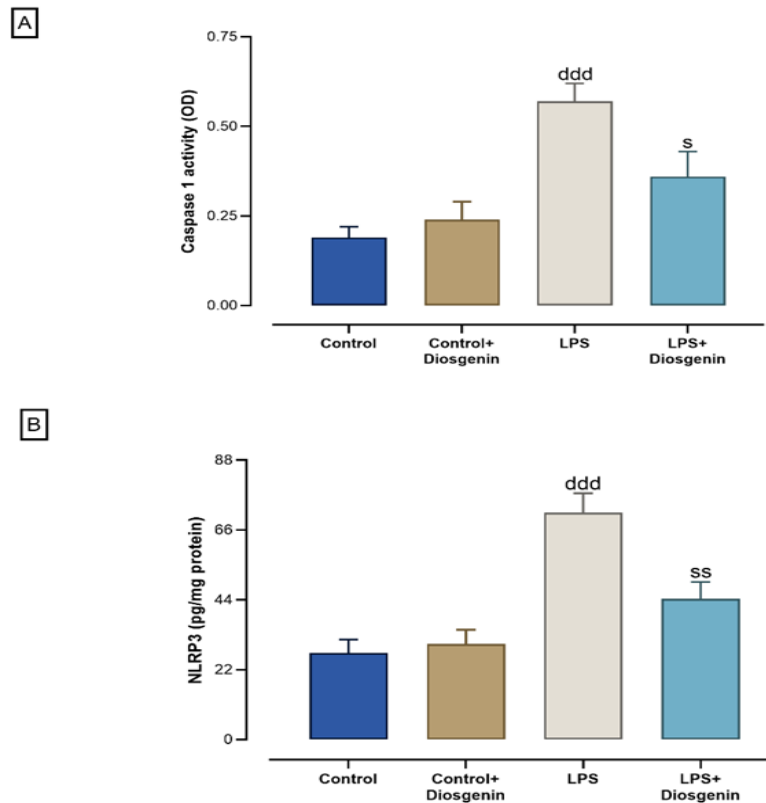


Figure 4. Effects of diosgenin on pyroptosis-related indices following LPS challenge. Renal level of caspase-1 (A) and NLRP3 (B) in the studied groups. LPS markedly increased caspase-1 and NLRP3, indicating pyroptosis. Diosgenin pretreatment significantly decreased caspase-1 and NLRP3 levels. Data represent mean $\pm$ SEM. Statistical significance: ddd $p < 0.001$ vs. control; ss $p < 0.01$ and s $p < 0.05$ vs. LPS.

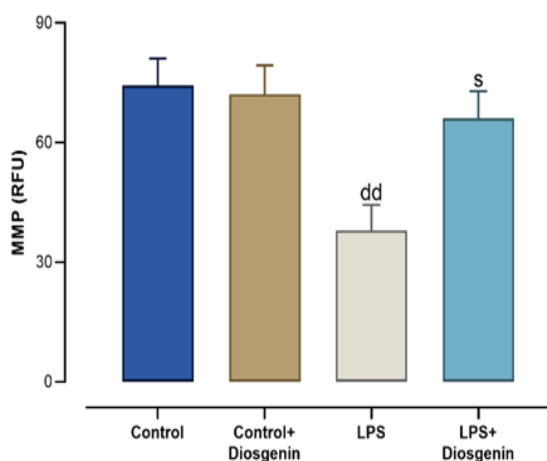


Figure 5. Effects of diosgenin on mitochondrial membrane potential (MMP) following LPS challenge. Renal level of mitochondrial membrane potential (MMP) in the studied groups. LPS markedly reduced MMP, indicating mitochondrial dysfunction. Diosgenin pretreatment significantly restored MMP. Data represent mean $\pm$ SEM. Statistical significance: dd $p < 0.01$ vs. control; s $p < 0.05$ vs. LPS.

Discussion

AKI is a complex and multifactorial syndrome, the onset and progression of which are closely linked to nephrotoxicity, ischemia-hypoxia, dysregulated inflammatory responses, alterations in glomerular hemodynamics, and the aberrant activation of autophagy, apoptosis, and oxidative stress pathways (Guo et al. 2019). LPS, commonly referred to as endotoxin, is a major structural component of the outer membrane of Gram-negative bacteria. Accumulating experimental and clinical evidence indicates that systemic exposure to LPS elicits robust inflammatory responses and plays a critical role in the development of sepsis and sepsis-associated acute kidney injury (Wang et al. 2023; Wu et al. 2025). In the present study, LPS was employed to establish an experimental model of AKI in

mice, with serum levels of blood urea nitrogen (BUN), creatinine (Cr), and cystatin C (CysC) used as validated and complementary indicators of renal dysfunction (Kamel et al. 2025). Consistent with this approach, systemic LPS challenge resulted in marked renal functional impairment, as evidenced by significant elevations in serum BUN, Cr, and CysC at 24 hr post-administration compared with the control group, thereby confirming successful induction of AKI. These findings are in line with well-established experimental models of LPS-induced AKI, in which elevations in BUN and creatinine reflect reduced glomerular filtration capacity and impaired handling of nitrogenous waste (Stasi et al. 2025; Xu et al. 2026). However, accumulating evidence indicates that serum Cr is an insensitive marker in the early phases of AKI due to its delayed rise following renal injury and its dependence on non-renal determinants such as muscle mass and volume status (Waikar and Bonventre 2009). In this context, serum CysC has emerged as a more sensitive and reliable biomarker of early renal dysfunction, as it is freely filtered by the glomerulus and is far less affected by extrarenal factors such as muscle mass, age, and sex compared with serum creatinine (Inker et al. 2012; Shlipak et al. 2013). Accordingly, the concurrent elevation of serum markers of renal dysfunction further supports the robust establishment of renal dysfunction at the evaluated time point. As stated by Tran et al., following systemic exposure of mice to LPS at a dose of 10 mg/kg, at least 24 hr is required for serum Cr and BUN to rise to diagnostically significant levels, thereby providing a reliable functional window for mechanistic investigations of LPS-induced AKI (Tran et al. 2011). Collectively, these indices offer a solid functional framework for subsequent analyses of oxidative stress, inflammation, and apoptosis.

In our hands, diosgenin treatment of LPS-challenged mice markedly lowered BUN, Cr, and CysC, pointing to substantial

renal protection. This aligns with the 2024 report by Jia et al., who found that diosgenin protected against membranous glomerulonephritis induced by cationic bovine serum albumin in mice, curbing oxidative stress and renal inflammation via NF- κ B downregulation and thereby preserving glomerular structure (Jia et al. 2024). Moreover, Chiang et al. (2024) reported that diosgenin confers protection against ischemia/reperfusion-induced kidney injury by safeguarding renal proximal tubular epithelial cells through suppression of NADPH oxidase 4 (NOX4) activation and inhibition of ROS Production, thereby limiting downstream inflammation, fibrosis, and apoptosis and significantly reduced renal MDA levels and attenuated oxidative stress by modifying NOX4/P65 signaling pathways (Chiang et al. 2024).

Oxidative stress, arising from excessive generation of ROS that exceed the capacity of endogenous antioxidant defense systems, is recognized as a key pathogenic mechanism in LPS-induced AKI (Liang et al. 2022). According to accumulating evidence, systemic exposure to LPS rapidly provokes excessive ROS production which contributes to redox imbalance and the development of sepsis-associated renal complications (Farhang-Nasab et al. 2025; Li et al. 2021). In this research study, LPS challenge markedly increased renal levels of MDA and nitrite, both indicative of enhanced oxidative stress and nitrosative stress, while simultaneously suppressing the activity of endogenous antioxidant enzymes such as SOD. These alterations collectively demonstrate the establishment of pronounced oxidative imbalance in renal tissue. Consistent with our findings, Liu et al. in 2015 reported that LPS challenge in murine models induced a pronounced oxidative and nitrosative stress response in renal tissue, as evidenced by elevated levels of MDA and nitric oxide, accompanied by decreased activity of the endogenous antioxidant enzyme SOD (Liu et al. 2015). Conversely, diosgenin treatment attenuated

oxidative stress in the present study. Although renal nitrite was elevated following LPS challenge, diosgenin produced a non-significant reduction, suggesting its primary antioxidant effect targets lipid peroxidation rather than nitrosative stress.

Inflammation represents a central mechanism in the pathogenesis of AKI, particularly in sepsis-induced models. Following LPS exposure, a robust activation of the innate immune system occurs, leading to the overexpression and release of key inflammatory mediators, including IL-1 β , IL-6, and TNF- α are observed (Wang et al. 2025). In line with these reports, the observed data in our study clearly confirmed the initiation of an inflammatory response within the renal tissue following LPS administration. Numerous studies have confirmed that pro-inflammatory cytokines play a central role in the pathogenesis of LPS-induced AKI (Chong et al. 2024; Shi et al. 2022). In our study, the elevated level of MPO, NLRP3, IL-17 in renal tissue following LPS administration confirmed the establishment of a pronounced inflammatory milieu. Emerging evidence suggests that IL-17 is an important contributor to the pathogenesis of sepsis-associated acute kidney injury. Produced predominantly by T-helper 17 (Th17) lymphocytes, $\gamma\delta$ T cells, and innate lymphoid cells, IL-17 amplifies inflammatory responses through induction of chemokines and cytokines that promote neutrophil recruitment and activation within injured renal tissue. Increased IL-17 signaling has been associated with enhanced oxidative stress, activation of NF- κ B-dependent inflammatory pathways, and exacerbation of tubular epithelial cell injury during endotoxemia and sepsis. Furthermore, recent studies have demonstrated that IL-17 may interact with inflammasome-related signaling pathways, thereby contributing to sustained inflammatory injury and progression of acute kidney dysfunction (Elrashidy et al. 2025; More et al. 2025). Therefore, the

significant elevation of renal IL-17 observed following LPS administration in the present study is consistent with its recognized pathogenic role in septic AKI. Importantly, the ability of diosgenin to suppress IL-17 levels may represent an additional mechanism contributing to its renoprotective and anti-inflammatory effects (Biswas 2018; Ge et al. 2020; Jin et al. 2023). Diosgenin-treated, LPS-challenged mice in our study (40 mg/kg) showed significantly lower renal cytokine content overall, supporting the idea that anti-inflammatory activity accounts for part of diosgenin protective effect against LPS-induced AKI. In line with our data, anti-inflammatory effect of diosgenin effectively inhibited inflammatory responses via inhibition of the NF- κ B signaling pathway (Jia et al. 2024). Chiang et.al in 2024 demonstrated that diosgenin exerted an anti-inflammatory effect via reducing the expression of inflammatory proteins, including IL-1 β , IL-6, and TNF- α in the hypoxia-stimulated renal tubular epithelial cells (Chiang et al. 2024).

Pyroptosis is considered a novel form of inflammation-related cell death in kidney cells. The most extensively characterized mechanism of pyroptosis is mediated by the NOD-like receptor protein 3 (NLRP3) inflammasome, a multiprotein complex composed of NLRP3, the adaptor protein apoptosis-associated speck-like protein containing a CARD (ASC), and the effector protease caspase-1 (Chen et al. 2025a). Extensive evidence indicates that aberrant activation of the NLRP3 inflammasome plays a pivotal role in the development and progression of kidney injury, including chronic kidney disease (De Vriese et al. 2025), calcium oxalate nephrocalcinosis (Xu et al. 2025), diabetic nephropathy (Chen et al. 2025b), and AKI. Moreover, mitochondrial dysfunction has been widely recognized as a central mechanistic driver of NLRP3 inflammasome activation and pyroptosis in renal injury through mitochondrial reactive oxygen species generation and release of mitochondrial

damage-associated molecular patterns (Wu et al. 2025). In this study, we found that LPS induced the formation of the NLRP3 inflammasome and stimulated caspase-1. Consequently, therapeutic agents capable of attenuating both inflammation and pyroptosis are considered promising candidates for the prevention and treatment of AKI (Chen et al. 2025a; Li et al. 2025). While diosgenin reversed the effect of LPS that activated NLRP3-mediated pyroptosis. Furthermore, in our study, diosgenin significantly reduced the levels of NLRP3 and caspase-1 and attenuated kidney injury under sepsis conditions. In line with our findings, a portion of the renoprotective effect observed with diosgenin administration may be attributed to its anti-pyroptotic activity. Diosgenin, a natural steroidal sapogenin, has previously been shown to inhibit pyroptosis in renal injury models by modulating key regulators such as the Nrf2/NLRP3 pathway, thereby reducing caspase-1 activation, GSDMD cleavage, and inflammatory cytokine release (Shanshan et al. 2025; Tang et al. 2024). These findings support the notion that diosgenin exerts its nephroprotective effects, at least in part, by suppressing pyroptosis in renal tubular cells under inflammatory and oxidative stress conditions.

In conclusion, the findings of the present study indicate that diosgenin significantly attenuates LPS-induced renal injury in mice and is associated with reduced oxidative stress, reduced IL-17-related inflammatory responses, decreased NLRP3/caspase-1 signaling, and preservation of MMP. These observations suggest that diosgenin exerts renoprotective effects in experimental sepsis-associated AKI. Nevertheless, further mechanistic studies are warranted to clarify the precise molecular pathways involved. Based on the present findings, diosgenin may represent a promising adjunctive strategy for mitigating kidney injury under septic conditions.

Acknowledgment

None.

Conflicts of interest

No conflicts of interest are declared by the authors.

Ethical Considerations

The study was conducted in accordance with institutional ethical requirements for the care and use of laboratory animals.

Code of Ethics

IR.SHAHED.REC.1396.58.

Authors' Contributions

Z.M: Investigation, Methodology, Formal analysis, Validation, Data curation, Writing – original draft, Writing – review & editing. A.S: Investigation, Methodology, Formal analysis, Validation, Data curation, Writing – original draft, Writing – review & editing. M.R.: Conceptualization, Supervision, Resources, Funding acquisition, Investigation, Methodology, Project administration, Formal analysis, Validation, Data curation, Writing – original draft, Writing – review & editing.

Abbreviations

AKI, Acute Kidney Injury .BUN, Blood Urea Nitrogen .Cr, Creatinine; CysC, Cystatin C; DAMP, Damage-Associated Molecular Pattern; GSDMD, Gasdermin D; IL-1 β , Interleukin 1 beta; IL-6, Interleukin 6; IL-17, Interleukin 17; IL-18, Interleukin 18; LPS, Lipopolysaccharide; MAPK, Mitogen-Activated Protein Kinase; MDA, Malondialdehyde; MMP, Mitochondrial Membrane Potential; MPO, Myeloperoxidase; NF- κ B, Nuclear Factor kappa-light-chain-enhancer of activated B cells; NLRP3, NOD-like receptor protein 3 Nrf2, Nuclear factor erythroid 2-related factor 2; RFU, Relative Fluorescence Units; ROS, Reactive Oxygen Species; SOD, Superoxide Dismutase; TLR4, Toll-Like Receptor 4; TNF- α , Tumor Necrosis Factor alpha

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